

Of Mice and Men:

Tracking the Origins of Metastatic Prostate Cancer

In most cases, prostate cancer is a treatable disease. Typically slow growing tumors that occur in men at a median age of 70 years are often treated effectively by interfering with androgen hormone signaling. But in 10 percent of cases, prostate cancers metastasize, become resistant to androgen deprivation therapy, and turn lethal. Kathleen Kelly, Ph.D., Chief of CCR's Cell and Cancer Biology Branch, has a long-standing interest in understanding the transformation from normal prostate cells into primary cancer and then into metastatic disease. Led by a desire to identify the earliest origins of prostate cancer, Kelly turned to a model system that allows her to study the cells that give rise to the disease as well as trace its metastatic spread.

Cancer's Original Sin

Prostate tumors initially require androgen hormone signaling to survive, so androgen-deprivation therapy (ADT), using drugs that inhibit androgen-receptor signaling, has been a highly effective therapeutic option for many patients. Over the years, the drugs that can inhibit androgen receptor signaling have improved such that the time between when the prostate cancer patient is treated and when he succumbs to the disease has increased. But, when the cancer progresses, it is almost always linked to the development of androgen-independence or “castrate-resistant” prostate cancer. Metastasis is invariably associated with a castrate-resistant form of the disease.

“When I first started thinking about the source of metastases,” said

Kelly, “one of the things I found very interesting about the healthy prostate is that when you take away androgen, the prostate shrinks and involutes. And when you add androgen back, it grows.” There aren't very many dividing cells in the normal prostate, but manipulations of androgen provided a striking demonstration of the existence and importance of androgen-independent stem cells in the healthy prostate.

“One of the ideas in the field that hasn't been proven or disproven yet, is that in prostate cancer, an immature undifferentiated cancer cell ultimately gives rise to resistance and metastases,” said Kelly. “The hypothesis is that this cancer stem cell doesn't require—or has unique mechanisms for obtaining— androgen receptor signaling, so it survives androgen-deprivation therapy.”

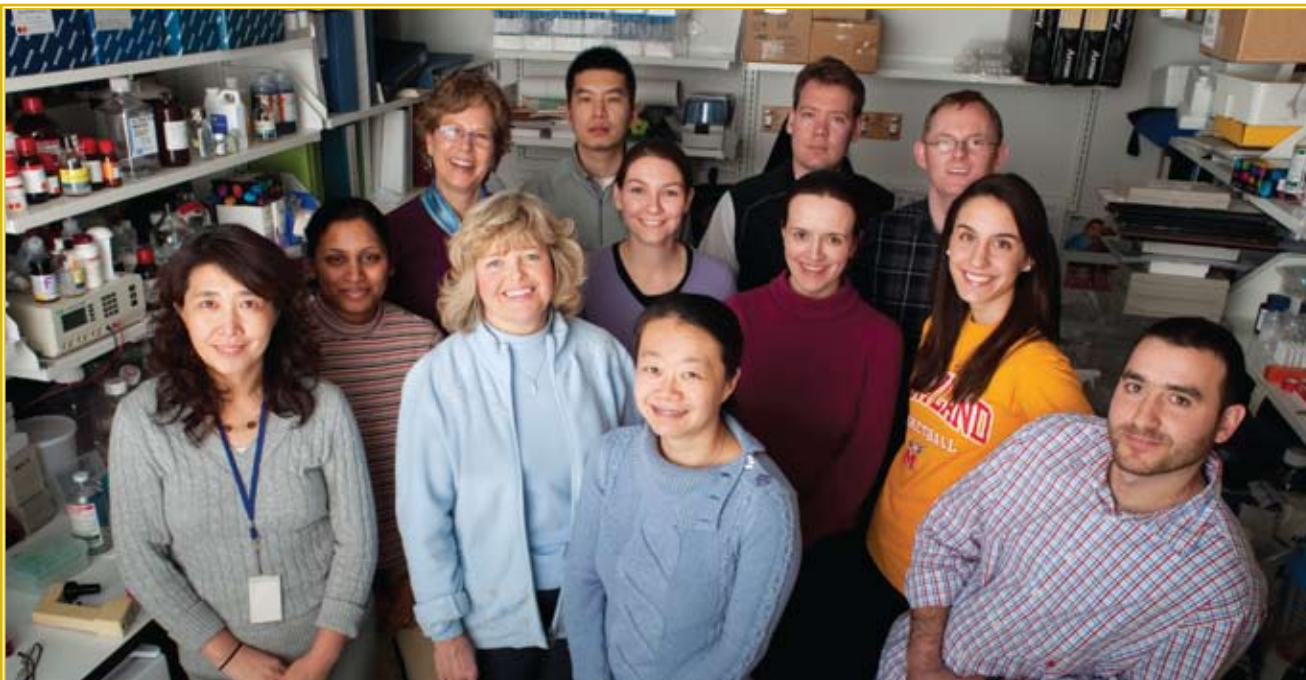
To test the hypothesis, Kelly wanted to look at the cells that initiate prostate cancers and follow their progression.

Building a Better Model

Many human cancers can be effectively studied through xenografts, in which primary tumor cells are injected into mice with compromised immune systems so that they do not reject the foreign cancer cells. Prostate cancer is unusual in that it is extremely difficult to reproduce in such a model system. Furthermore, although they can be kept alive, primary prostate tumor cells do not thrive in culture conditions.

There are a handful of prostate cancer cell lines, which were mostly derived from metastases and not primary tumors, so they have multiple mutations and have been in culture for

(Photo: R. Baer)



Kathleen Kelly, Ph.D., and members of her laboratory.

many years. Thus, they are problematic as a tool to study the properties of cancer stem cells, as they might not exist in a living organism.

"The approach I decided to take was a mouse model," said Kelly. "I chose an aggressive model, because I thought there was a higher chance we could study a metastatic process."

Kelly, with her graduate student, Philip Martin, D.V.M., made a mouse with deletions of two tumor suppressors—*PTEN* and *TP53*—in prostate epithelial cells. Mutations of *PTEN* and *TP53* occur at fairly high frequency in human populations and are often associated with aggressive, castrate-resistant, and metastatic disease.

In addition to the two genetic deletions, Kelly and Martin introduced a light-emitting reporter gene—luciferase—which allowed tracking of transplanted cells that carried the genetic deletions.

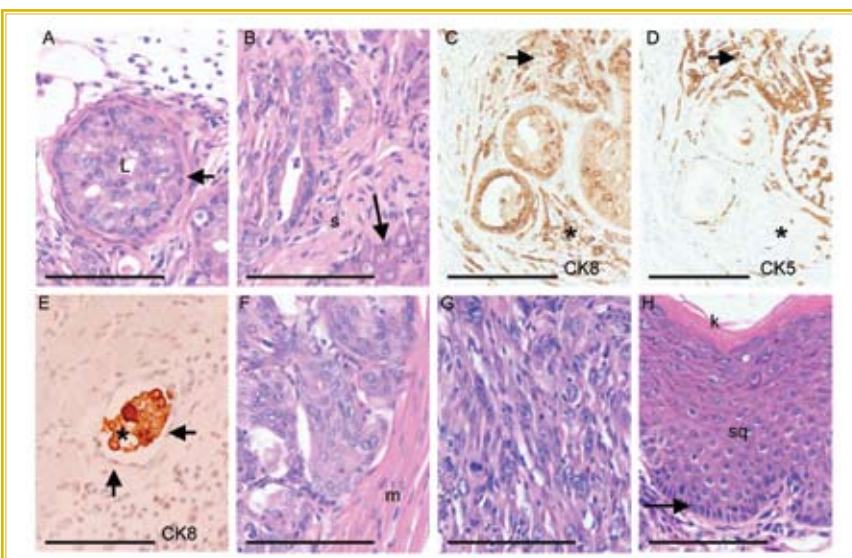
"The idea we had was that we would be able to sort through the tumor cells and find the tumor-initiating cells in these mice," said Kelly, "but first we had to fully characterize our model." So using his training in veterinary pathology, Martin led a full longitudinal study of their mouse model. (See "The Veterinary Perspective.")

"One of the most important things we were able to show is that, unlike other mouse models of prostate cancer, this one produced cells with metastatic potential," said Martin. The mice rapidly developed tumors composed of multiple cell types, which were lethal in approximately seven months.

Panning for Cells

In the Kelly lab, Research Fellow Wassim Abou-Kheir, Ph.D., has studied the progenitor cells undergoing transformation in the prostate of these genetically modified mice. He used selective culturing conditions to study the self-renewing capabilities of prostate cells extracted from these

(Image: K. Kelly, CCR)



Kelly and her colleagues have created a mouse model of prostate cancer with tumors that display a diversity of tumor types: (A) prostatic intraepithelial neoplasia; (B) adenocarcinoma; (C) and (D) prostate tumors positively stained for two epithelial filament markers (arrow: CK8 and CK5) or only one (asterisk: CK8 but not CK5); (E) vascular invasion; (F) prostatic urothelial carcinoma; (G) sarcomatoid carcinoma; (H) basal/squamous carcinoma.

mice. He found that the number of progenitor cells in these mice was strongly amplified and that the cells had a greatly increased ability to self-renew compared to cells from normal mice. "They can be cultured indefinitely and will continue as progenitors. We believe that the tumor-initiating cells are within this self-renewing population," said Kelly.

Meanwhile, at a nearby bench, Research Fellow Paul Hynes, Ph.D., is searching for tumor-initiating cells by teasing apart identifiable cells from the primary prostate tumors in these mice. The process of fractionating the cells involves separating them out on the basis of protein markers on their cell surface. "He's finding that there is an undifferentiated tumor-initiating

cell that can give rise to both basal and luminal cells," said Kelly, referring to the two major cell types in the prostate.

The team has also found such bipotential progenitor cells in cell lines that they have created from single tumor cells, i.e., clonal cell lines, and analyzed. These cells are also immature, can give rise to both basal and luminal cells, and metastasize when grafted into the mouse prostate.

The Veterinary Perspective

Photo: R. Baer



Philip Martin, D.V.M., and Kathleen Kelly, Ph.D.

In 2003, Mark Simpson, D.V.M., Ph.D., Head of the Comparative Molecular Pathology Unit of CCR's Laboratory of Cancer Biology and Genetics, launched a new training initiative under the NIH Graduate Partnership Program titled the Comparative Molecular Pathology Research Training Program. His aim was to provide opportunities for veterinarians to gain postdoctoral training in pathology and human biomedical research. The program operates with multiple NIH institute intramural programs and university partners, with training leading to a Ph.D. and eligibility to certify as a specialist in veterinary pathology.

"We recognized that an incredible set of advancements were forthcoming from the genomic revolution and the sequencing of the human genome," said Simpson. "But understanding the function of most genes requires their study in the context of the biology of a model organism, that is, an animal."

A veterinary pathologist, Simpson knew that this training would support a bridge between molecular discovery and whole animal pathophysiology. "What was needed was to bring more veterinarians to the NIH and provide them with the opportunity to get research training in human biomedical research," said Simpson. "Training alongside medical and basic scientists fosters a shared vocabulary and approaches to translational research."

Philip Martin, D.V.M., arrived at the NIH in 2005, part way through a residency in veterinary pathology. "The program was perfect for me because it allowed me to fulfill my residency requirements, including the national board certification exam, as well as pursue a Ph.D., which is important for pathologists who are interested in animal models of human disease."

Martin is currently finishing up his dissertation in the laboratory of Kathleen

Kelly, Ph.D., but, in the meantime has accepted a full-time position as a pathologist at CCR's Center for Advanced Preclinical Research (CAPR) in Frederick, Md. CAPR is dedicated to improving preclinical evaluation for effective cancer diagnosis and treatment.

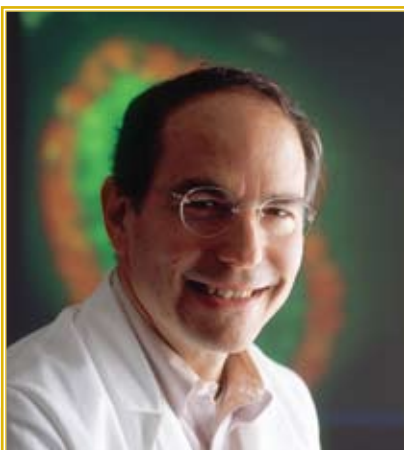
"If we're going to study mice as models of human disease, we need to be sure that it is a relevant form of disease," explained Martin. "The need to analyze the pathology is extremely important. Many models, upon rigorous pathological examination, are not the type of cancer that the investigators were hoping to study."

"Having Philip's perspective in the lab has really helped me understand the strong and weak points of mouse models," said Kelly.

Simpson hopes that this initiative will contribute not only to prevention and treatment of disease in humans, but also in animals. "There's a special perspective we bring because of our orientation to disease in multiple species." By integrating the comparative perspective with human biomedical research, Simpson aims to train D.V.M./Ph.D. scientists who are capable of leading research collaborations at the forefront of scientific discovery.

For more information about the Comparative Molecular Pathology Research Training Program, please visit <http://ccr.cancer.gov/resources/training/applications/programInformation.asp>.

(Photo: R. Baer)



Peter Choyke, M.D.

"We are really interested in determining how tumor-initiating cells are related to the different cell lineages and then understanding what their response is to androgen and androgen deprivation," said Kelly.

Kelly and her team have found that some of these clonal cell lines are sensitive to androgen deprivation, but there is a component of cells that does survive androgen deprivation. Kelly noted that in a very recent paper from Memorial Sloan Kettering Cancer Center, researchers also found cells from human prostate cancer-derived cell lines that have tumor-initiating capacity and are insensitive to androgen.

"From our mouse work, and now this human cell line, it does appear that a relatively undifferentiated tumor-initiating cell will lead to luminal adenocarcinoma of the prostate," concluded Kelly.

Understanding more about the cells responsible for driving tumor formation will provide new insights into how to more effectively diagnose potentially progressive disease and target these specific populations therapeutically.

Tracking the Spread

Another challenge for prostate cancer research lies in the differences in androgen sensitivity that result when cells are removed from the environment of the organism. "It's complicated to take apart the

response," said Kelly, noting that cells are just not as sensitive to androgen deprivation in culture.

Among the clonal cell lines that Kelly's team has generated from their mouse model, a few behave like human prostate cancer cells in that they are androgen-sensitive and give rise to adenocarcinoma. "So we are looking at metabolically labeling and tracking these cells in the mouse," said Kelly.

Peter Choyke, M.D., Program Director of the Molecular Imaging Program at CCR, runs both preclinical and clinical imaging facilities. "Our goal is to develop molecular imaging tools that are translatable into people with the hope that we can diagnose, stage, or monitor cancer patients in a noninvasive way over time," said Choyke. Among the tools in his research armamentarium is a micro-positron emission tomography (PET) scanner for use on small animals.

Choyke and Kelly are planning to use PET to look at metabolic changes in prostate cancer cells relative to normal tissue, both as a tool to better understand prostate cancer progression in the whole organism and as a means to improve the ability to image these cancer cells in men.

"There's a lot of interest in the metabolomics of cancer and, from an imaging perspective, one of the interesting aspects of prostate cancer is that, early on, it is not particularly well visualized by the standard PET scan," explained Choyke. Whereas most tumors differentially depend on glucose uptake, prostate cancers early in development do not, and PET scanning relies on cells taking up radiolabeled glucose (18F-fluorodeoxyglucose or FDG).

"As the prostate cancer advances and becomes more malignant, it starts to take up more FDG. So, there is some kind of glucose utilization switch that occurs later in its development," said Choyke.

From studies that Kelly has conducted on prostate cancer cells,

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she has additional reasons to believe that prostate cancer cell metabolism is altered during the progression of the disease. In addition to changes in glucose metabolism, she believes that changes in fatty acid metabolism might also be important, and that early prostate cancers differentially utilize fatty acids. Choyke and Kelly plan to study this by using PET to monitor uptake of the fatty acid precursor, 11C-acetate, which corresponds to the activity of an enzyme that synthesizes fatty acids.

Specifically, Kelly will use a model in which their clonal cell line is introduced into the prostates of mice that have been castrated and implanted with testosterone pellets. Subsequent removal of the pellets will mimic androgen-deprivation therapy and result in the development of androgen-independent malignancies.

"We want to know whether a marker of fatty acid metabolism—11C-acetate—would be a sensitive way of finding prostate cancer cells either in a primary state or following androgen deprivation," explained Kelly.

One of the biggest challenges to studying and treating any metastatic disease is being able to find and track the cancer as it spreads. Ultimately, Kelly hopes that studying this progression in a carefully characterized and controlled mouse model will provide insights to address that challenge in man.

To learn more about Dr. Kelly's research, please visit her CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=kellyk>.

To learn more about the Molecular Imaging Program at CCR, please visit Dr. Choyke's CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?Name=choyke>.